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AIDS Testing

A Comprehensive Guide to Technical, Medical, Social, Legal, and Management Issues

Second Edition

Foreword by Walter R. Dowdle

With 59 Illustrations

Springer-Verlag
New York Berlin Heidelberg London Paris
Tokyo Hong Kong Barcelona Budapest

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FDA Regulation of HIV-Related Tests and Procedures

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The U.S. Food and Drug Administration (FDA) regulates drugs and medical devices under the Federal Food, Drug and Cosmetic (FD&C) Act (21 U.S.C. §§ 301 *et seq.*). Medical products may also be subject to the licensing provisions of the Public Health Service (PHS) Act (42 U.S.C. §§ 201 *et seq.*) when they meet the definition of a biologic product. Biologic products additionally are regulated either as drugs or medical devices under the FD&C Act and Amendments. To date, the FDA has regulated all human immunodeficiency virus (HIV)-related tests as biologic products, except that the Agency has regulated sample collection systems used in conjunction with HIV-related tests as medical devices. Test procedures provided by clinical laboratories currently are regulated by the Health Care Financing Administration under the Clinical Laboratory Improvement Amendments of 1988 (CLIA '88) (42 U.S.C. §§ 201 note, 263a, 263a note).

For drugs, biologic products, and class III medical devices, the purpose of regulation by the FDA is to determine the safety and efficacy (or effectiveness) of these medical products prior to their commercialization and to protect the public from adulterated and misbranded products. To accomplish its mandate, the Agency reviews information regarding the manufacturing, clinical performance, and labeling of the product. Manufacturers must demonstrate that products are manufactured consistently and in conformance with standards set forth in published Current Good Manufacturing Practice Regulations (cGMPs), that they have been found to be safe and effective for their intended use by current scientific methods including well controlled clinical trials, that all medical claims have been validated, and that the products are properly labeled.

For HIV-related diagnostic tests, safety concerns have included control of biohazards during the manufacturing process, safety during clinical use of kits containing components that have undergone virus inactivation, and the welfare of test subjects with regard to proper interpretation and use of test results. Efficacy concerns have focused on determination of test

sensitivity, specificity, and reproducibility in actual intended-use settings. As with other in vitro diagnostic tests, HIV-related tests may be intended for use in epidemiologic surveillance, screening, and medical diagnosis or prognosis. The application of HIV tests as screening tests for HIV antibodies to determine the suitability of blood donors, and hence the safety of blood products, has been closely regulated.

Elements of FDA Regulation

The FDA exercise of statutory authorities over HIV-related tests involves three levels of control: (1) approval of product and of establishment license applications and amendments (now called supplements); (2) surveillance; and (3) enforcement.

Because it is illegal to distribute commercially any unapproved biologic or drug products for clinical use or to promote them for unapproved indications, manufacturers must obtain exemptions to engage in clinical studies. The FDA reviews Investigational New Drug (IND) or Investigational New Device Exemption (IDE) applications to evaluate consistency of product manufacturing, safety and efficacy based on preclinical data, suitable credentials of the investigators, adequacy of the clinical study design, and approval of the study by an Institutional Review Board (IRB).

After clinical investigation, manufacturers of test kits regulated as biologic products must apply for and receive separate licenses for the product and for the establishment where it is manufactured before they engage in commercial distribution. Establishment License Applications are reviewed to evaluate the facility's design including systems validation, assess personnel management and training, and ensure compliance with cGMPs, including equipment validation, quality control procedures, and record keeping. An Environmental Assessment, as specified in 21 CFR 25.1, is also evaluated. Product License Applications are reviewed for validation of manufacturing procedures, including monitoring of the identity, purity, potency, stability, and consistency of components; for validation of product claims through preclinical and clinical studies; and for completeness and accuracy of product labeling. A prelicense inspection is conducted while manufacturing is ongoing as part of the review process. After approval of establishment and product applications, changes in facilities, manufacturing procedures, and product labeling are reviewed through submitted license amendments (supplements). Guidance concerning the content of applications is provided in the FDA's draft of "Points to Consider in the Manufacture and Clinical Evaluation of In-Vitro Tests to Detect Antibodies to the Human Immunodeficiency Virus, Type 1 (1989)," [available from Food and Drug Administration, Center for Biologics Evaluation and Research, Division of Congressional and

Consumer Affairs, HFM-12, Woodmont Office Center, Suite 200N, 1401 Rockville Pike, Rockville, MD 20852-1448 (telefax no. 301-594-1938)].

After a product has been approved, the Agency monitors performance through various surveillance mechanisms, including periodic inspections at least biennially. The Agency may ask that the manufacturer conduct "Phase IV" studies to investigate product performance issues as a condition of approval. The FDA audits mandatory reports of errors, accidents, and adverse reactions (or Medical Device Reports), which may document product failures. Furthermore, the Agency may monitor test kits on a lot-by-lot basis through a release mechanism. Since they were first licensed in 1985, the FDA has performed lot release testing prior to distribution for all lots of HIV tests that are approved for blood donor screening.

The Agency also may exercise control through enforcement actions. It may invoke a variety of administrative and judicial remedies to guard against statutory and regulatory violations, including license suspensions and revocations under the PHS Act, product recalls and seizures, and civil or criminal proceedings under the FD&C Act.

HIV-Related Tests Currently Regulated by the FDA

Since 1985 the FDA has approved a variety of HIV-related tests based on HIV virus antigens or antibodies directed toward these antigens (or both). Some of the currently available tests are approved for use in blood donor screening and medical diagnosis (Table 4.1), and others are approved primarily for use in medical diagnosis (Table 4.2). These tests are characterized as HIV antibody screening tests, supplemental tests used to verify the results of antibody screening tests, and tests for HIV antigen(s). Most approved screening tests for HIV-1 use standard indirect enzyme-linked immunosorbent assay (ELISA) technology with an enzyme-labeled "second antibody," although one test uses an enzyme-labeled antigen in an "antibody sandwich" design. The antigenic substrates have included whole viral lysates from cell culture, purified viral proteins, recombinant DNA-derived viral proteins, and synthetic peptides. Two rapid tests have also been approved: a latex agglutination test and an enzyme immunoassay based on antigen-coated microparticles. Supplemental tests for antibodies to HIV-1 include Western blot and immunofluorescence assays. The approved test for HIV-1 antigen detects viral p24 antigen in a capture ELISA format and includes use of a blocking antibody to identify true-positive results. The FDA has licensed one screening test for HIV-2 antibodies and two screening tests for combined detection of antibodies to HIV-1 plus HIV-2. For each licensed test kit, the criteria used by the FDA to validate the test are provided in a "Summary of Basis for Approval" document [available from the Food and Drug Administration,

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TABLE 4.1. HIV test kits currently approved for blood donor screening and medical diagnosis.

HIV screen	Manufacturer	Product trade name	Date licensed
HIV-1 antibodies	Abbott Laboratories, North Chicago, IL	HIVAB HIV-1 EIA	3/1/85
	Cambridge Biotech Corp., Worcester, MA	Recombigen (*env* and *gag*) HIV-1 EIA	5/1/90
		MicroTrak HIV-1 EIA (*env* and *gag*)	5/30/90
	Cellular Products, Buffalo, NY	Retro-Tek HIV-1 ELISA	8/29/86
	Genetic Systems Corp., Redmond, WA	Genetic Systems LAV EIA	2/18/86
	Organon Teknika Corp., Durham, NC	HIV-1 Bio-EnzaBead	4/5/85
		Vironostika HIV-1 MicroElisa System	12/18/87
	Ortho Diagnostic Systems, Raritan, NJ	Ortho HIV-1 ELISA Test System	2/21/86
	United Biomedical, Hauppauge, NY	UBI-Olympus HIV-1 EIA	5/31/89
HIV-2 antibodies	Genetic Systems Corp., Redmond, WA	Genetic Systems HIV-2 EIA	4/25/90
HIV-1 and HIV-2 antibodies	Abbott Laboratories, North Chicago, IL	HIVAB HIV-1/HIV-2 (rDNA) EIA	2/14/92
	Genetic Systems Corp., Redmond, WA	Genetic Systems HIV-1/HIV-2 EIA	9/25/91

TABLE 4.2. HIV test kits currently approved primarily for medical diagnosis.

Purpose of test	Manufacturer	Product trade name	Date licensed
Rapid screen for HIV-1 antibodies	Cambridge Biotech Corp., Worcester, MA	Recombigen HIV-1 LA Test[a]	12/13/88
	Murex Corp., Norcross, GA	SUDS HIV-1 Test[a]	5/22/92
Detection of HIV-1 antigen	Abbott Laboratories, North Chicago, IL	HIVAG-1	8/3/89
Supplemental test for HIV-1 antibodies	Bio-Rad Laboratories, Hercules, CA	Novapath HIV-1 Immunoblot	6/15/90
	Cambridge Biotech Corp., Worcester, MA	HIV-1 Western Blot Kit	1/3/91
	Epitope, Beaverton, OR	EPIblot HIV-1 Western Blot Kit	3/20/91
	Waldheim Pharmazeutika, Vienna, Austria	Fluorognost HIV-1 IFA[a]	2/5/92

[a]The test may be used for blood donor screening only when routine ELISA tests are unavailable or impractical.

Freedom of Information Staff, HFI-35, 5600 Fishers Lane, Room 12A-16, Rockville, MD 20857 (telefax no. 301-443-1726)].

Regulatory Concerns Regarding Screening Tests

The Agency has regarded test sensitivity as the leading issue when giving approval for screening tests for antibodies to HIV-1 or HIV-2. Strategies for evaluating test kit sensitivity have included comparisons with research tests and previously licensed tests. Preclinical studies have used dilutional series of positive human sera as well as seroconversion series to show equivalence to a "state of the art" performance standard such as a Western blot assay. Clinical trials have used "head to head" comparisons with reference tests in high risk populations. Claims for sensitivity have been based on the percent positivity for randomly selected AIDS patients, although reporting of comparative analysis with other test methods has been permitted in test kit package inserts. To ensure that commercial kits continue to meet a uniform minimum standard for sensitivity, the FDA developed and has maintained a lot release panel of sera that is used to test every lot of antibody test kits prior to distribution.

A continuing controversy surrounds the inconsistency of test kits for detection of IgM antibodies to HIV. Most test kit manufacturers have avoided the use of IgM-specific conjugates in the indirect ELISA design because of the familiar problem of false-positive reactions with non-specifically "sticky" IgM antibodies. Such a problem has been observed for several blood donor screening ELISA tests following immunizations for influenza.[1] Despite early skepticism, there is little doubt that increased sensitivity for IgM can enhance antibody detection during the first few weeks after HIV infection. The "antibody sandwich" ELISA was developed in part to improve sensitivity for IgM.

The validation of tests using recombinant and synthetic antigens of HIV as substrates has presented special challenges. A leading concern is the theoretic possibility that rapid genetic evolution of HIV worldwide could result in the emergence of infectious virus variants eliciting an antibody response that could escape detection by a narrow range of epitopes represented in the antigenic substrate. To address this possibility, the FDA has required that test kits based on neoantigens be validated in premarket studies against approximately 1000 known positive sera, including samples from all of the known major regions of the epidemic. Additionally, some manufacturers have been asked to conduct phase IV surveillance studies to look for false-negative results due to virus variation. To determine the appropriate level of scientific concern, on December 18, 1992 the Agency discussed with its Blood Products Advisory Committee the issue of genetic variation of HIV in the immunodominant region of the viral envelope. The gene sequence in this region is the basis for use of synthetic peptides as HIV antigenic substrates.[2] After presenta-

tions of worldwide data, the committee recommended that that FDA approve such products when they meet current standards, but that they continue to monitor virus variation.

Another current issue concerns the validation of tests using samples other than conventional serum or plasma. Testing of serum eluted from spots of capillary blood dried onto filter paper was developed to improve neonatal screening for HIV exposure—as a surveillance tool for monitoring the spread of the epidemic in childbearing women and as a way to identify HIV-exposed children. Three ELISA screening tests have been approved by the FDA for such a procedure. The possibility of testing for HIV antibodies present in samples of urine and oral fluid (gingival transudate) has been pursued. Despite the reduced levels of antibodies in these samples, detection methods have been developed that approach the clinical sensitivity of detection in blood. The advantages of noninvasive sample collection must be weighed carefully against the possibility of reduced test sensitivity for determining the public health implications of these test systems.

The accuracy of rapid tests that require subjective interpretation by the operator is closely linked with training. Poorly trained operators can easily misinterpret test results. The Agency has attempted to address this problem, in cooperation with manufacturers, through labeling to ensure adequacy of instructions for use and by asking manufacturers to provide training in the performance and interpretation of the test. Proficiency monitoring of test performance also is a responsibility of clinical laboratories under CLIA '88 implementing regulations.

The approved test for HIV antigen is labeled "for intended use in medical diagnosis (including prognosis) and for monitoring HIV expression in tissue cultures." The efficacy of screening blood donors for HIV antigen was investigated in a large multicenter trial conducted in 1989.[3] Based on the results and a discussion at a meeting of the FDA's Blood Products Advisory Committee on March 23, 1989, tests for HIV antigen are not recommended for blood donor screening owing to the lack of a demonstrated public health benefit. This position may have to be reexamined if antigen tests with greater sensitivity are developed. Research tests for HIV antigen that utilize procedures to dissociate immune complexes have increased the sensitivity for detection of HIV antigen in the presence of antibodies. In theory, these tests may offer an advantage for monitoring antiviral therapy and as diagnostics in the perinatal setting, but they have not been shown to improve routine screening.

Regulatory Concerns Regarding Supplemental Tests

Since 1985 it has been a policy of the U.S. Public Health Service to recommend that confirmatory testing be performed prior to notification of test subjects concerning their screening test results for HIV. This

strategy permits confident identification of true-positive test results and minimizes the impact of reporting false-positive results, which occur inevitably with large-scale screening programs. By far the most widely used procedure for this purpose is the Western blot assay, which is based on electrophoretically separating proteins of the whole virus. Immunofluorescence tests based on HIV-infected and uninfected control cells are less widely used, probably because of the special requirements for equipment and training. The FDA has termed these "additional, more specific tests" to stress their positive predictive value while recognizing the possibility that they, like screening tests, are not definitive when negative. Licensed Western blot test kits for HIV-1 antibodies have been available since 1987, and a licensed HIV-1 immunofluorescence test kit became available in 1992. Validation of these tests has emphasized the need for sensitivity comparable to the approved screening tests and for accuracy of a positive test interpretation. For Western blots, reproducibility of the banding pattern also has been examined closely. These tests are subject to lot release control using the same FDA panel that is applied to screening tests.

The greatest concern regarding Western blot tests has been the high prevalence of nonspecific banding patterns, resulting in indeterminate test results. Unfortunately, this phenomenon is intrinsic to the technology due to a variety of causes that include antibodies in the patient sample that bind to nonviral proteins in the viral antigen preparation and antibodies of unknown specificity that cross-react with viral proteins. Attempts to optimize test performance based on interpretive criteria have necessitated a choice between maximizing sensitivity or specificity. The criteria recommended for the first FDA-approved test were conservative in that they required a major band from each gene group of HIV structural proteins: *gag* (p24), *pol* (p31), and *env* (either gp41 or gp120/160). These criteria maximized test specificity but at the expense of sensitivity in some clinical settings, including early seroconversion and acquired immunodeficiency syndrome (AIDS). Currently used criteria are less stringent and have resulted in tests that are more sensitive but have a higher false-positive rate. The FDA has accepted product amendments for revised Western blot interpretive criteria that are consistent with the prevailing scientific view and the recommendations of the Public Health Service. The agency has attempted to reduce the occurrence of falsely reactive bands by close attention to quality control issues in manufacturing and through lot release criteria. There are future prospects for technology improvements that may further reduce the existing problem of nonspecific bands.

Food and Drug Administration approval of an HIV-1 immunofluorescence test has provided clinical laboratories with an alternative to the Western blot test. Care must be taken to ensure the proficiency of the operator, who must be highly skilled in recognizing both the pattern and

the intensity of fluorescence to achieve a correct test interpretation. To address this need, the manufacturer provides materials and educational training for operator qualification and proficiency monitoring as part of the approved product.

The FDA has become increasingly concerned about the commercialization of unlicensed supplemental test kits. The Agency did not take enforcement action against the use of unlicensed tests for a brief period after 1985 owing to the lack of availability of licensed tests and the pressing public health need for a means to validate HIV screening test results. As licensed supplemental tests now have become widely available, the Agency intends to initiate enforcement actions against unlicensed products. The Agency also intends to initiate enforcement action to effect compliance in such areas as the use of alternative samples (e.g., blood spot eluates, oral fluid, and urine) in conjunction with screening and supplemental tests.

Sample Collection Systems for HIV-Related Testing

Many sample collection systems are regulated as class I medical devices, which do not require premarket approval. However, sample collection systems intended for use in HIV-related testing have been regarded as class III medical devices that require premarket approval. Basically, these systems fall into two categories: products intended only for professional use in medical care settings and products intended for lay use. FDA concerns common to both types of product include (1) the adequacy of the instructions for sample collection, storage, and shipping; (2) validation of the adequacy of the sample and its compatability with a licensed screening and supplemental test; and (3) performance of the test by a properly certified clinical laboratory, including proficiency monitoring. For tests intended only for professional use, FDA reviews whether labeling and marketing limit distribution to a health care setting and if a mechanism for reporting results through a health care provider is present.

The FDA reviews collection systems intended for lay use for validation of control of potential biohazards in the home use setting, comprehensibility of the instructions by lay persons, a means of assessing the adequacy of sample collection, and adequacy of the pretest and posttest education and counseling. Additionally, the FDA seeks evidence that sample collection and testing outside a health care setting is in the interest of the public's health. This question has generated much controversy each time it has been brought before an FDA Advisory Committee for public discussion. The FDA's current position is that demonstration of safety for lay use should include a determination that public health benefits outweigh any risks.

FDA Perspective on Emerging Technologies

Despite the excellent performance characteristics of existing tests for HIV, medical and scientific needs continue to accelerate the pace of new technology development in HIV-related diagnostics, especially to improve early detection and to provide results with higher specificity. These needs include improvement in perinatal diagnosis, more sensitive blood donor screening tests, screening and supplemental tests for HIV-2, more cost-efficient test systems, and tests for lay use. Novel tests for HIV disease staging, prognosis, and therapy monitoring may also emerge. For example, research tests for the level of viral expression, the degree of immune compromise, and resistance to antiviral drugs have been developed. Some of these tests may be based on analytes other than HIV components or anti-viral antibodies.

Tests based on amplification of viral gene segments using the polymerase chain reaction or other methods have already been introduced in some clinical laboratories and are under development as commercial products. In clinical studies, gene-based tests have shown promise for development in a variety of uses such as diagnosis in high risk settings (perinatal exposure, acute viral syndrome of HIV) and potentially as supplemental tests following routine screening for antibodies. The FDA's concerns when reviewing applications for these tests focus on consistency in manufacturing and on the manufacturer's validation of well defined clinical claims. The potential use of these tests as the primary test for mass screening presently is limited by practical constraints (e.g., low through-put and the labor-intensive nature of the test) and concerns over the potential for false-positive results due to sample or laboratory contamination. In addition, there is a well recognized need for reagent standards and quality assurance systems to maintain the proficiency of laboratories performing testing by these methods.

Advances in supplemental testing may take a variety of forms. Scientists at the Centers for Disease Control and Prevention (CDC) have developed a modified Western blot procedure that may improve the consistency of that assay.[4] In addition, studies have been done to develop the potential for accurate Western blot testing of blood spot eluates. Other developments may include supplemental test systems based on the use of synthetic peptide antigens and new immunofluorescence assays. FDA evaluates such developments in the context of requests for investigational exemption and new product applications or amendments (supplements); and it works cooperatively with manufacturers, especially in areas that address public health needs.

Considerations of economy and user convenience have led to the development of combined tests for HIV-1 and HIV-2. This trend, which is expected to continue, is facilitated by the ease with which neoantigens can be developed for addition to the existing antigenic substrate. For

instance, a manufacturer of an HIV-1 test may seek to develop a combination test by simple addition of an HIV-2 peptide or recombinant protein. In these cases, FDA routinely seeks validation that the modified kit has not lost sensitivity for HIV-1 and that it is as sensitive for HIV-2 as a test based on whole viral proteins of HIV-2. Reduced specificity has been a problem for the development of combination tests.

Conclusion

Regulation of HIV-related diagnostic tests by the FDA is intended to review consistency of manufacturing, validation of claims for intended use, and adequacy of labeling. Through the approval process, surveillance, and enforcement mechanisms, the FDA establishes and maintains manufacturing and product performance standards. This activity complements efforts of other government agencies to ensure proficiency standards in clinical laboratories. Particular discretion is exercised over approval of tests labeled for blood donor screening due to the direct effect of the accuracy of testing on the safety of blood products, which are themselves regulated as biologic products. Manufacturers of HIV-related diagnostic test kits must be aware of their obligations to comply with FDA regulations. Similarly, users of these kits should be aware that unapproved tests may violate the law. The process of FDA review and approval of new test technologies operates best with the cooperation of industry and the full participation of the medical community in developing and maintaining appropriate product standards.

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